PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Richard Voellmy

Appl. No:

Filed: October 26, 2001

Title: MOLECULAR REGULATORY CIRCUITS TO ACHIEVE SUSTAINED ACTIVATION OF GENES OF INTEREST BY A SINGLE STRESS

Grp: 1648

Examiner: Ulrike Winkler

CERTIFICATE OF MAILING

I hereby certify that this correspondance is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner of Patents, Washington, DC 20231

On Oct. 26, 01 R. Voellmy

Date Signature

Richard Voellmy

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents

Washington, D.C. 20231

Sir:

This is a continuation of application 09/304,121, which application claims priority from provisional application 60/084,236. Payment for five supernumerary independent claims and one multiple dependent claim has been included in the enclosed credit card payment form.

Please amend the above-identified continuation application as follows:

In the claims:

Please delete claims 1 - 8. Add claims 9 - 18:

9. A molecular regulatory circuit delivered into a cell for activation of a gene of interest by a single transient stress, comprising

(a) a gene for a transcription factor, the transcription factor being first expressed to a level sufficient to activate the gene of interest in response to a transient stress and thereafter activating its own expression and

(b) the gene of interest that is activated by the transcription factor.

10. A molecular regulatory circuit delivered into a cell for controlling the activity of a gene of interest by a single transient stress and a second stimulus, comprising

(a) a gene for a transcription factor, the transcription factor being first expressed to a level sufficient for activation of the gene of interest in response to a transient stress, acquiring activity in the presence of the second stimulus and thereafter activating its own expression and

(b) the gene of interest that is activated by the transcription factor in the presence of the second stimulus.

11. A method for activation of a gene of interest in a cell by a single transient stress, comprising

Delivering into the cell a molecular regulatory circuit comprising one or more nucleic acids comprising (a) a gene for a transcription factor that is first expressed to a level sufficient to activate the gene of interest in response to a transient stress

and thereafter activates its own expression and (b) the gene of interest that is activated by the transcription factor, and

Subjecting the cell containing the molecular regulatory circuit to a transient stress sufficient to cause expression of the transcription factor to a level sufficient to activate the gene of interest,

Thereby achieving sustained expression of the gene of interest.

12. A method for controlling the activity of a gene of interest in a cell by a single transient stress and a second stimulus, comprising

Delivering into the cell a molecular regulatory circuit comprising one or more nucleic acids comprising (a) a gene for a transcription factor that is first expressed to a level sufficient for activation of the gene of interest in response to a transient stress, acquires activity in the presence of the second stimulus and thereafter activates its own expression and (b) the gene of interest that is activated by the transcription factor in the presence of the second stimulus, and

Subjecting the cell containing the molecular regulatory circuit to the second stimulus and to a transient stress sufficient to cause expression of the transcription factor to a level sufficient to activate the gene of interest,

Thereby achieving sustained expression of the gene of interest.

13. The method of claim 12 further comprising the step of subsequently withdrawing the second stimulus, thereby achieving reduction of the activity or inactivation of the gene of interest.

14. A nucleic acid delivered into a cell comprising a gene for a transcription factor that is operably linked to a promoter activatable by a stress and by the transcription factor.

15. A nucleic acid delivered into a cell comprising a first gene for a transcription factor that is operably linked to a promoter activatable by stress and a second gene for the same transciption factor that is operably linked to a promoter activatable by the transcription factor.

16. A nucleic acid delivered into a cell comprising a gene for a transcription factor, the transcription factor being first expressed in response to a stress and thereafter activating its own expression.

17. A nucleic acid delivered into a cell comprising a gene for a transcription factor and a promoter that is functionally linked to the gene for the transcription factor such that the gene for the transcription factor is activatable by a stress and by the transcription factor.

18. The nucleic acid of any of claims 14-17, wherein the transcription factor is selected from the group consisting of a mutated heat shock transcription factor, a chimeric transcription factor, a constitutively active transcription factor and a transcription factor active in the presence of a second stimulus other than a stress.

PRELIMINARY REMARKS

To better protect what Applicant regards as its invention, Applicant wishes to characterize and claim the molecular circuits of the invention using functional language.

Support for claim 9 can be found in the sections of the specification that describe the operation of the three types of regulatory circuits of the invention and in the Figures relating to these sections:

In the type 1 circuits, the transcription factor is a mutated heat shock transcription factor (HSF). Note that, therefore, both transcription factor gene and gene of interest must be controlled by a stress-inducible promoter (the target promoter of the transcription factor). Thus, in the special case of the type 1 circuits, expression of the product of the gene of interest is not only stimulated by the transcription factor but also transiently occurs in response to an activating stress.

The operation of the circuits is described on p.12, lines 11-19 (See also Fig.1.): "In the cells, both genes [mutated transcription factor gene and gene of interest] are either silent or expressed at appropriately low levels in the absence of stress. When the cells are stressed, promoters in both elements are activated by endogenous HSF, which results in the expression and accumulation of the gene product of interest and of mutated HSF. Mutated HSF continues to activate transcription of both the gene of interest and its own gene, resulting in the synthesis of more product of interest and mutated HSF. This cycle continues even if the cells are no longer under stress. Consequently, the gene of interest will remain active until such time that the cell has exhausted its capacity to transcribe and translate nucleic acids."

When discounting the incidental (and not novel) feature specific to these circuits that the gene of interest can be transiently activated by a stress because it is under the control of a stress-inducible promoter, type 1 circuits are generically characterized as comprising a gene for a transcription factor, a mutated HSF, which transcription factor is first expressed to a level sufficient to activate the gene of interest in response to a transient stress. ("When the cells are stressed, promoters in both elements are activated by endogenous HSF, which results in the expression and accumulation of the gene product of interest and of mutated HSF. Mutated HSF continues to activate transcription of both the gene of interest and its own gene, resulting in the synthesis of more product of interest and mutated HSF.") The transcription factor activates thereafter its own expression. ("Mutated HSF continues to activate transcription of both the gene of interest and its own gene.") The circuits further comprise a gene of interest that is activated by

4-12. The latter example makes clear that the second stimulus can be a ligand whose presence is required for activity of the transcription factor. The operation of a type 3 circuit is described using the example of an EcR/RXR heterodimeric transcription factor whose activity requires the presence of a hormone ligand such as muristerone A. See p.22, lines 4-26 (partially reproduced below) and Fig.4. In the specific example, two sets of transcription factor subunit genes are given, one of which is regulated by a stress-inducible promoter and the other by a promoter responsive to the heterodimeric transcription factor. Note that the use of two transcription factor constructs instead of one in which the transcription factor gene(s) is activatable both by stress and the transcription factor is a variation of the circuits that is specifically discussed for type 3 circuits on p.21, lines 18-26 and Fig.3, and for type 2 circuits on p.21, lines 5-11.

P.22, lines 17-26: "This form of type 3 circuit operates as follows. In the absence of either one or both, stress and hormone, the gene of interest is silent. In the presence of hormone, a transient stress will activate one set of transcription factor genes, resulting in accumulation of EcR/RXR that is activated by hormone. Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest. Because the transcription factor is continually produced, activation of the gene of interest is sustained. Upon withdrawal of the hormone, transcription factor is inactivated, and the autoactivating loop is interrupted. The gene of interest is no longer expressed, and the inactive transcription factor as well as protein of interest will eventually be degraded by intracellular proteolytic systems." Fig.4 illustrates the same mode of operation graphically.

Hence, a type 3 circuit is characterized as comprising genes for a transcription factor, here an EcR/RXR that is activated by a hormone (a special feature of the example circuit disregarded for the purpose of this general characterization, but see below), which factor is first expressed to a level sufficient for activation of the gene of interest in response to a transient stress. ("In the absence of either one or both, stress and hormone, the gene of interest is silent. In the presence of hormone, a transient stress will activate one set of transcription factor genes, resulting in accumulation of EcR/RXR that is activated by

hormone. Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest.") The transcription factor thereafter activates its own expression. ("Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest.") The circuits further comprise a gene of interest that is activated by the transcription factor. ("Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest.")

Fig.3 shows a second example type 3 circuit that involves a gene for a constitutively active transcription factor that is regulated both by stress and the transcription factor. Its operation is graphically depicted in Fig.3 and can be paraphrased as follows: upon a brief heat treatment (a transient stress), endogenous HSF1 is activated, and active endogenous HSF1 activates the gene for LexA-Act.Domain factor, which factor is then expressed and activates the cytokine gene (gene of interest) as well as activates its own expression. The second element of the circuit is a cytokine gene (gene of interest) that is activated by the transcription factor.

It should be clear from the above discussion of the functional characteristics of the different circuits of the invention as described in the specification that all circuits contain two types of elements, the functional interaction of which can be captured by the functional language present in claim 9:

(a) a gene for a transcription factor, the transcription factor being first expressed to a level sufficient to activate the gene of interest in response to a transient stress and thereafter activating its own expression and
(b) the gene of interest that is activated by the transcription factor.

Claim 10 is specific to the kind of type 3 circuit illustrated by the example on p.22 of the specification. In this kind of type 3 circuit the transcription factor is a transcription factor that is active in the presence of a second stimulus (other than a stress). See p.21, lines 17-18 and the example on p.22, lines 4-12. The example makes clear that the second

stimulus of the circuit can be a ligand whose presence is required for activity of the transcription factor. The example transcription factor is an EcR/RXR heterodimeric transcription factor whose activity requires the presence of a hormone ligand such as muristerone A. See p.22, lines 4-26 (partially reproduced below) and Fig.4. In the specific example, two sets of transcription factor subunit genes are given, one of which is regulated by a stress-inducible promoter and the other by a promoter responsive to the heterodimeric transcription factor. Note that the use of two transcription factor constructs instead of one in which the transcription factor gene(s) is activatable both by stress and the transcription factor is a variation of the circuits that is specifically discussed for type 3 circuits on p.21, lines 18-26 and Fig.3, and for type 2 circuits on p.21, lines 5-11.

P.22, lines 17-26: "This form of type 3 circuit operates as follows. "In the absence of either one or both, stress and hormone, the gene of interest is silent. In the presence of hormone, a transient stress will activate one set of transcription factor genes, resulting in accumulation of EcR/RXR that is activated by hormone. Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest. Because the transcription factor is continually produced, activation of the gene of interest is sustained. Upon withdrawal of the hormone, transcription factor is inactivated, and the autoactivating loop is interrupted. The gene of interest is no longer expressed, and the inactive transcription factor as well as protein of interest will eventually be degraded by intracellular proteolytic systems." Fig.4 illustrates the same mode of operation graphically.

Thus, the circuit comprises a gene for a transcription factor (a set of two genes for subunits of the factor in the specific example). The transcription factor encoded by the genes is first expressed to a level sufficient for activation of the gene of interest in response to a transient stress and acquires activity in the presence of the second stimulus. ("In the absence of either one or both, stress and hormone, the gene of interest is silent. In the presence of hormone, a transient stress will activate one set of transcription factor genes, resulting in accumulation of EcR/RXR that is activated by hormone.") Thereafter, the transcription factor will activate its own expression. ("Active factor will activate

expression from the second set of transcription factor genes as well as the gene of interest.") The circuit further comprises a gene of interest that is activated by the transcription factor in the presence of the second stimulus. ("Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest. Because the transcription factor is continually produced, activation of the gene of interest is sustained. Upon withdrawal of the hormone, transcription factor is inactivated,...")

Claim 10 uses the above functional language to describe the interaction between the two types of elements of the above-discussed form of a type 3 molecular circuit:

> A molecular regulatory circuit for controlling the activity of a gene of interest in a cell by a single transient stress and a second stimulus, comprising
> (a) a gene for a transcription factor, the transcription factor being first expressed to a level sufficient for activation of the gene of interest in response to a transient stress, acquiring activity in the presence of the second stimulus and thereafter activating its own expression and
> (b) the gene of interest that is activated by the transcription factor in the presence of the second stimulus.

Claim 11 recasts the composition of matter claim 9 as a method claim. The molecular circuits of the invention are described in language identical to that in claim 9. It is obvious that the method comprises the two steps of first delivering the circuit and then activating it. See, for example, p.12, lines 10-13. Claim 12 is a similar method claim that relates to the use of the kind of type 3 circuit captured by claim 10. Again, the molecular circuit is described in language identical to that in claim 10. The second step includes the additional element of subjection of the cell containing the circuit to the second stimulus. Support for this element can be found on p.22, lines 18-22. Claim 13 is dependent on claim 12 and claims the additional step of withdrawal of the second stimulus for inactivation of the circuit. Support for this latter step is provided on p.22, lines 23-30.

Claim 16 is a composition of matter claim directed to the novel transcription factor element of the molecular circuits. The same functional language is used to characterize this composition that was used to characterize the molecular circuits in claim 9. Claim 17 is directed to the same composition, making more restricted use of functional language. Claims 14 and 15 are also directed to the same matter using language identical to that used to characterize the molecular circuits in claims 1 and 9 of the parent application 09/304,121 (see claim 1). Claim 18 is dependent on the latter independent claims. Support for the elements of this claim can be found in the specification. For "mutated heat shock transcription factor (HSF)" see p.12, lines 8, 14, 16, and p.14, lines 14, 17. For "chimeric transcription factor" see the definition on p.9, lines 3-6, and the examples on p.19, lines 15-20, p.20, lines 21-28 (Note that the factors discussed are specifically referred to as "chimeras" on line 27.), and p.21, lines 21-23. For "constitutively active transcription factor" see p.21, lines 17-18. Finally, for "a transcription factor active in the presence of a second stimulus other than a stress" see the example on p.22, lines 4-9 and line 20. For the characterization of the second stimulus as being different from a stress, see the sentence bridging pp.21 and 22.

Applicant believes that, when coupled with a disclaimer of the term extending beyond that of the patent to issue from parent application 09/304,121 to avoid an obviousness type double patenting rejection, the claims proposed in the present continuation application are in condition for allowance.

If the Examiner believes that a telephone conversation would aid his/her review, the Examiner is kindly invited to call Applicant (who is his own attorney) at (305) 243-5815.

Respectfully Submitted,

Richard Voellmy

Reg. # 40,859